IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Sommadossi *et al.*	Confirmation No.:	5135
Serial No.:	10/715,729	Art Unit:	1648
Filed:	November 17, 2003	Examiner:	Humphrey, Louise W. Z.
For:	2'-BRANCHED NUCLEOSIDES AND FLAVIVIRIDAE MUTATION	Attorney Docket No: (CAM:	11874-064-999 417451-999064) IDX 1022

DECLARATION UNDER 37 C.F.R. 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, David Standring, Ph.D., declare and state that:

1. I have 26 years of experience in the field of virology, including 16 years of anti-viral research related to the treatment of hepatitis C virus ("HCV") infections.

2. I have received my doctorate degree in bioorganic chemistry from Harvard University in 1980. I performed post-doctoral research at the Department of Biochemistry & Biophysics at the University of California, San Francisco from 1980 to 1982.

3. I have served as an Assistant Research Biochemist and Assistant Professor at the University of California, San Francisco from 1982 to 1994; Group Leader, Hepatitis in the Virology Department at Bristol-Myers Squibb Research Institute (Wallingford, CT) from 1994 to 1998; Research Fellow and Associate Director in the Virology Department at Schering Plough Research Institute (Kenilworth, NJ) from 1998 to 2000; Executive Director of Research at Novirio Pharmaceuticals, Inc. from 2000 to 2002; Vice President of Biology and Senior Vice President of Biology at Idenix Pharmaceuticals, Inc from 2002 to 2007.

4. I currently hold the position of Executive Vice President of Biology at Idenix Pharmaceuticals, Inc. Idenix Pharmaceuticals, Inc. is a co-owner of the above-captioned application. A copy of my curriculum vitae is attached as **Exhibit 1**.

5. I am a co-inventor in the above-captioned patent application.

6. I have reviewed the specification of the above-captioned application, a copy of which is attached as **Exhibit 2**. I have read the claims of the above-captioned application as rejected by the United States Patent and Trademark Office ("USPTO"), a copy of which is attached as **Exhibit 3**. I am familiar with the Examiner's comments regarding the alleged obviousness of the claims of the above-captioned application in the Office Actions dated December 23, 2008 and July 7, 2009, copies of which are attached as **Exhibit 4**.

7. **The Arens reference.** Based on my personal experience and on my review of Arens *et al., J. Clin. Virology*, 22: 11-29 (2001) ("Arens"), the reference cited by the USPTO in **Exhibit 5**, it is my opinion that specific mutations relevant to HCV drug resistance are unpredictable, due at least in part to the high genetic heterogeneity of HCV and the high error rate of viral replication. While Arens provides one way to determine genotypic changes that appear in response to a given drug, such changes will include random variations having no connection with drug resistance. Moreover, some genotypic changes may impart drug resistance directly, while other changes may work by indirect mechanisms such as compensatory changes that increase the replication of a replication-deficient mutant. Yet other changes seen in the HCV replicon system may simply be adaptive changes that help the HCV replicon to propagate in tissue culture. None of this information is predictable, nor is it taught by Arens.

8. Furthermore, it is my opinion that Arens provides no information on the phenotypic consequences of a mutation in the context of HCV. Thus, the idea that any specific mutant, not to mention a HCV mutant that results from exposure to a 2'-C-methyl nucleoside polymerase inhibitor (*i.e.*, the S282T mutant discussed below), is highly replication-impaired and therefore potentially more susceptible to a second agent from another class of drugs is not at all evident from Arens. Indeed, the data

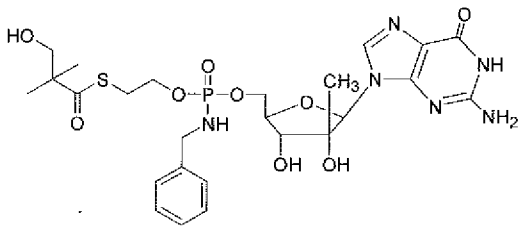
provided in paragraph 13 below demonstrate that certain mutations that appear to increase the replication of the virus provide no obvious resistance to the drug, and may even enhance the ability of the virus to survive subsequent treatment with other antiviral agents.

9. Thus, in my opinion, there is a substantial difference between a genotypic change of unknown function and relationship to drug resistance, and a phenotypic change shown to confer resistance to a specific drug but with a high price to the virus in terms of replication capacity and the ability to be resistant to a second agent. The first case has no implication for therapy, while the second case, as demonstrated by the data in paragraphs 12-15 below, has significant therapeutic implications for HCV therapy.

10. **The Carroll patent.** Based on my review of U.S. Patent No. 7,105,499 to Carroll ("Carroll"), cited by the USPTO in **Exhibit 6**, it is my opinion that Carroll merely provides a general teaching of combination therapy for HCV. As has been long understood, combination therapy provides benefits in that (i) the activity of the combination is often enhanced (*e.g.*, additive or synergistic antiviral effect) compared to the monotherapy, and (ii) agents of different classes are usually not cross-resistant. However, as shown in the data provided below, the resistance and replicative capacity of an individual HCV mutant varies widely as compared to the wild-type virus. This is not apparent from the teachings of Carroll.

11. **HCV Mutant Replicon Study.** A study to evaluate the resistance profile of HCV polymerase inhibitors was performed under my supervision at Idenix Pharmaceuticals, Inc., Cambridge, MA ("the replicon study"). The *in vitro* antiviral activity of HCV polymerase inhibitors was evaluated against a series of mutant HCV replicons, some of which are known to be resistant to HCV polymerase or protease inhibitors, including 2'-C-methyl nucleosides.

12. The S282T mutant replicon is a HCV replicon known to be resistant to 2'-C-methyl nucleosides. In the replicon study, the resistance of S282T to 2'-C-methyl nucleosides was confirmed using a HCV polymerase inhibitor of formula A ("Compound A"):



(A)

13. As shown below, S282T was the only HCV mutant replicon in the replicon study to confer viral resistance Compound A.

Replicon	Confers resistance to[a]	EC_{50}[b] +/- SD	N[c]
S282T	2'-C-Methyl-nucleosides	23.4 +/- 6.7	6
C316Y	Benzofurans/benzothiadiazines	0.63 +/- 0.33	5
S365T[d]	Benzofurans	1.70 ± 0.48	3
M414T	Benzothiadiazines	0.82 ± 0.23	4
M423T	Thiophenes	0.89 ± 0.26	4
M423V	Thiophenes[p]/Pyranoindoles	1.10 ± 0.27	3
A442T	Pyranoindoles	0.86 ± 0.09	3
I482L	Thiophenes	0.75 ± 0.27	4
P495L[d]	Benzimidazole-based	1.13 ± 0.33	4
I585T	None known	1.82 ± 0.61	3

a. Resistance profile based on published reports.
b. Compound A.
c. Number of replicates.

14. The replication capacity of individual HCV mutant replicons was tested in the replicon study, including S282T. Replication capacity of the individual HCV mutants varied substantially as compared to wild-type virus. Three mutants replicated at or above wild-type capacity: M414T (122% ± 40%), C445F (196% ± 48%) and I585T (239% ± 86%). Four mutants replicated at an intermediate level relative to wild-type: C316Y (29% ± 9%), M423T (40% ± 10%), A442T (60% ± 7%) and I482L (73% ± 22%). Six mutants replicated at a low level relative to wild-type: S282T (5% ± 2%), S365T (2% ± 1%), M423V (11% ± 3%), C445Y (14% ± 6%), Y448H (10% ± 5%) and P495L (16% ± 5%).

15. In my opinion, mutant replicons that replicate above the capacity of the wild-type virus, *e.g.*, M414T, C445F and I585T, are unlikely to show any advantage in therapeutic response to combination therapy with a 2'-C-methyl nucleosides and a second antiviral agent. In contrast, it is my opinion that a highly compromised HCV mutant such as S282T, which replicates at 5% the capacity of the wild-type virus, is an attractive target for combination therapy with a 2'-C-methyl nucleoside and one or more antiviral agents.

16. In my opinion, the above-described variation in replication capacity of the individual HCV mutant replicons tested could not have been anticipated or predicted. In my opinion, one would not have been able to predict that the replication capacity of a specific 2'-C-methyl nucleoside mutant replicon, S282T, would be as highly compromised as demonstrated in the replicon study above.

17. **HCV Cross-Resistance Study**. A second study was performed under my supervision at Idenix Pharmaceuticals, Inc. in order to assess cross-resistance in the S282T mutant ("the cross-resistance study"). Compound B is a non-nucleoside HCV polymerase inhibitor that has been shown to bind to a location (the palm site[1]) distinct from the region in the NS5B HCV polymerase in which 2'-C-methyl nucleosides bind (the active site).[2]

18. In the cross-resistance study, Compound B was tested for cross-resistance to the S282T mutant replicon. The activity of Compound B against S282T was statistically equivalent to activity against the wild-type HCV virus, demonstrating no substantial cross-resistance between Compound B and 2'-C-methyl nucleoside polymerase inhibitors such as Compound A:

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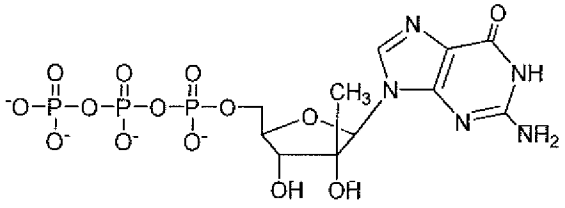
[1] Bilello, J.P. *et al.*, *Hepatology*, 2008, 48(Supp.): 1166A, copy attached as **Exhibit 7**.

[2] Thompson, P.A. *et al.*, *Hepatology*, 2008, 48(Supp.): 1164A, copy attached as **Exhibit 8**.

Wild-type/Replicon	Mean EC_{50} +/- SD (μM)	N[a]
Wild-type	1.53 +/- 0.63	23
S282T	2.68 +/- 0.77	5

a. Number of replicates.

19. In the cross-resistance study, Ribavirin was tested for cross-resistance to the S282T mutant replicon. The *in vitro* antiviral activities of ribavirin and Compound C, the phosphorylated active metabolite of Compound A, were evaluated in S282T mutant HCV NS5B enzymes. Compound C has the formula:



(C)

20. The S282T mutant polymerase was less susceptible to inhibition by Compound C by a factor of ten. In contrast, ribavirin triphosphate exhibited almost a twenty-fold enhancement in activity against the S282T mutant polymerase:

Wild-type/ Replicon	Mean IC_{50} +/- SD (μM)		N[a]
	Ribavirin-TP[b]	Compound C	
Wild-type	883.4 +/- 378	0.27 +/- 0.06	3
S282T	45.9 +/- 16.2	2.61 +/- 0.72	3

a. Number of replicates.
b. "TP" = triphosphate.

21. The lack of cross-resistance and enhanced activity of ribavirin to the S282T mutant HCV replicon was confirmed in the cross-resistance study using a cell-based assay. Two cell lines resistant to Compound A, "Cell line A" and "Cell line DR," bearing the S282T signature mutation were tested for cross-resistance. Cell line A was shown to be 30-fold less susceptible to treatment with Compound A. Cell line DR was shown to be 130-fold less susceptible to Compound A. Ribavirin, which is weakly active against wild-type HCV replicon (EC_{50} = 38 +/- 2.8 μM), demonstrated 2.2 and 3.2 greater activity against Cell line A and Cell line DR,

respectively. Thus, Ribavirin was not cross-resistant to S282T mutant HCV cell lines, and even demonstrated enhanced antiviral activity against HCV strains harboring an S282T mutation.

22. **Conclusions.** 2'-C-methyl nucleoside therapy results in highly compromised HCV mutants which in my opinion are unforeseeably susceptible to combination therapy with other anti-viral agents which act by a different mechanism. The above data, which demonstrate that Ribavirin and Compound B, which attack HCV by mechanisms distinct from that of a 2'-C-methyl nucleoside, do not show cross-resistance to 2'-C-methyl nucleoside therapy in the HCV mutant S282T and may potentially show even better activity against this variant due to its impaired replication. Therefore, in my opinion, combination therapy of a 2'-C-methyl nucleoside and a second drug that acts at a location other than the region in the NS5B HCV polymerase in which 2'-C-methyl nucleosides bind provides a potentially superior method of HCV treatment not appreciated by Arens or Carroll.

23. I, David Standring further declare that all statements made herein are of my own knowledge to be true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent that may issue there from.

David Standring

DAVID STANDRING

JAN 4th, 2010

Date